Trägermaterial zur simultanen Bindung genotypischer und phänotypischer Substanzen

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Abstract of **DE4237381**

Described is a support material that can simultaneously bind both genotypic and phenotypic substances. The respective areas, which can have surface-modifying matter such as anionic exchangers and/or affinity ligands, simultaneously bind, for example, nucleic acids and proteins or peptides. The support materials described can be used in processes for evolutive optimization of biopolymers, wherein genotype and phenotype can be bound at the same time so as to furnish them for further analysis.

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(54) Trägermaterial zur simultanen Bindung genotypischer und phänotypischer Substanzen

(57) Es wird ein Trägermaterial beschrieben, das simultan sowohl genotypische Substanzen als auch phänotypische Substanzen binden kann. Die entsprechenden Areale, die oberflächenmodifizierte Stoffe, wie Anionenaustauscher und/oder Affinitätsliganden aufweisen kann, bindet simultan beispielsweise Nukleinsäuren und Proteine oder Peptide. Die beschriebenen Trägermaterialien können in Verfahren zur evolutiven Optimierung und Biopolymeren eingesetzt werden, wobei gleichzeitig Genotyp und Phänotyp gebunden werden, um diese einer weiteren Analyse zuzuführen.

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(57) Abstract

The invention relates to a process for the purification of a substance with magnetic particles treated with a reagent which binds the particles. After a binding reaction in a first medium, the particles and the substance bound to them are separated and transferred to a second medium. According to the invention, a surface tension releasing agent is dispensed at least the mediums before the particles are separated from it. This promotes the complete collection of the particles.

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PURIFICATION PROCESS USING MAGNETIC PARTICLES

Background of the invention

The present invention relates to the purification of biological substances using magnetic particles which bind the material specifically from a mixture. The invention can be used for instance for purifying nucleic acids DNA or RNA.

Magnetic particles can be coated with a separation reagent which reacts specifically with a desired biological substance. The particles and the bound substance are separated from the mixture and thereafter the substance is released from the particles for further prosecution. Nowadays this is done in practice so that the particles are drawn with a magnet against the wall of the vessel containing the mixture, and the liquid is poured or sucked off the vessel. Thereafter a new liquid can be dispensed into the vessel. Manual or automatic equipments for such separation technology are also commercially available (e.g., Spherotec, Inc., AutoMag Processor (USA), Merck Magnetic Rack (Darmstadt, Germany), PerSeptive Biosystems 96 well plate separator, Multi-6 Separator, Solo-Sep Separator (USA), Dynal Magnetic Particles Concentrators).

The old purification technique for DNA involves ultracentrifugation in a dense cesium chloride gradient. However, also magnetic particle technology described above has been used for purifying nucleic acids.

WO 94/18565 (Labsystems Oy) suggests a method and device for magnetic particle specific binding assay, in which magnetic particles are separated from a mixture by a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel and the rod is pulled in its upper position, whereby the particles are released. Thus all steps of the assay can be carried out in a separate vessel without having to transfer liquids. In the last vessel, a measurement is carried out.

WO 96/12959 (Labsystems Oy) suggests a magnetic particle transfer tool comprising an elongated body with a concavely tapered tip part. The body further comprises means for providing a longitudinal magnetic field to collect particles to the tip of the body. The magnetic field can be eliminated in order to release the

particles. This tool can be used especially for collecting particles from a large volume and releasing them into a very small volume.

General description of the invention

Now a method according to claim 1 has been invented. Some preferable embodiments of the invention are defined in the other claims.

According to the invention, material to be purified is dispensed in a first medium containing magnetic particles, which have been coated with a binding reagent for the material. The binding reaction takes place, after which the particles are separated by means of a magnetic probe and transferred into a second medium, in which a desired further reaction necessary for the purification may take place. The particles can be transferred similarly via further mediums for carrying out further steps of the purification process. All the vessels may contain the necessary reaction medium ready when the particles are transferred into it. Preferably the particles are also released from the probe in the second and subsequent mediums.

According to the invention, at least one of the mediums contains a surface tension releasing agent. This promotes the complete collection of the particles.

Some of such agents have been used also before in this connection to promote the binding of the substance to be separated, see e.g. Wipat et al., Microbiology (1994), 140, 2067. In these known methods, the particles are not transferred from a vessel to another bu they are held on the wall of the vessel by means of a external magnet, while the medium is removed from the vessel.

The invention can be used especially for purifying nucleic acids, such as ssDNA, dsDNA, and mRNA.

Brief description of the drawings

The enclosed drawings form a part of the written description.
Figure 1 shows the effect of a detergent in collecting and releasing steps of magnetizable particles.
Figure 2 shows the effect of salt and saccharose in collecting and releasing buffer.
Figure 3 shows the effect of protein in collecting and releasing buffer.
Figure 4 shows the effect of a detergent when magnetic particles of different suppliers were used.

Detailed description of the invention

The invention can be used for instance for the purification of cells, viruses, subcellular organelles, proteins, and especially nucleic acid materials.

The magnetic particles are preferably paramagnetic. The size of the particles is usually less than 50 μm, preferably 0.1 - 10 μm, and most preferably 1 - 5 μm. The concentration of the particles may be eg. 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.

The particles have been coated or treated with a binding reagent, eg. silicon, lectins and/or other reactive functional groups such a oligonucleotides, antibodies, antigens, streptavidin, or biotin.

The particles are preferably transferred from a vessel to another by using a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel, and when the rod is pulled to its upper position the particles are released.

Different kind of surface tension releasing compounds, especially water soluble compounds, can be used in the method. Examples of such are:

A. Tensides, such as
- Soaps
- Detergents; including anionic, kationic, non-ionic and zwitterionic compounds

B. Alcohols, such as
- Polyethylene and polyvinyl alcohols and their protein etc. derivatives

C. Proteins

D. Salts and carbohydrates in high concentrations, such as
- NaCl
- Saccharose

Also mixtures of compounds can be used.

Especially tensides such as detergents are suitable. Preferable detergents are ethoxylated anhydrosorbitol esters. The esters may contain eg. about 4 - 20 ethylene oxide groups.

The concentration of a tenside may be eg. 0.001 - 0.5% (w/v), preferably 0.005 - 0.1% (w/v), and most preferably 0.01 - 0.05% (w/v). The concentration of a protein

may be eg. 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v). The concentraion of a salt may be eg. 0.1 - 10 M, preferably 0.1 - 7 M.

For purification of DNA or mRNA from different sources (for instance, DNA from PCR amplification; DNA from blood, bone marrow or cultured cells; mRNA from eucaryotic total RNA or from crude extracts of animal tissues, cells and plants) the nucleic acids are immobilized by using magnetic particles. The binding can be mediated by the interaction of streptavidin and biotin, whereby particles coated with streptavidin and biotinylated DNA can be used. In addition, DNA can be adsorbed to the surface of the particles. The binding of mRNA can be mediated by Oligo $(dT)_{25}$ covalently coupled to the surface of the particles.

After the immobilization, the nucleic acids are washed several times to remove all the reaction components resulting from the amplification or other contaminants and, e.g., PCR inhibitors.

The washing can be performed by releasing and collecting complexes in a washing buffer and by transfering the complexes to another well containing fresh washing buffer.

For ssDNA purification the immobilized double-stranded DNA can be converted to a single-stranded by incubation with 0.1 M NaOH and using magnetic separation.

For the isolation of mRNA, it can be eluted from the particles by using a low salt buffer.

The purification process can be performed by a magnetic particle processor, in which all the mediums are ready in separate vessel. A surface tension releasing compound is preferably used in each medium. Suitable disposable plates, such as microtitration plates, comprising the necesary vessels can be used. In one plate, several parallel purifications can be accomplished.

Example of reagents used for a ssDNA purification

1. Particle suspension in eg. phosphate, Tris or Borate buffered saline, pH 7.4, containing 0.1% BSA, 15 mM NaN_3 and eg. 0.02% polyoxyethylene (20) sorbitan monolaurate (Tween 20 ™) as a surface tension releasing agent

2. Binding and Washing buffer (TEN): 10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, eg. 0.02% Tween 20 ™, 15 mM NaN_3, pH 7.5

3. TE buffer: 10 mM Tris-HCl, 1 mM EDTA, eg. 0.02% Tween 20 ™, 15 mM NaN_3, pH 7.5

4. Melting solution: 0.1 M NaOH, eg. 0.02% Tween 20 ™

5. eg. 0.02% Tween 20 ™ in distilled water, 15 mM NaN_3

Example of reagents used for a mRNA direct purification

1. Oligo $(dT)_{25}$ particle suspension in PBS, pH 7.4, containing eg. 0.02% Tween 20 ™ and 15 mM NaN_3

2. Lysis/binding buffer: 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT), 15 mM NaN_3, (eg. 0.02% Tween 20 ™)

3. Washing buffer with LiDS (SDS): 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS, 15 mM NaN_3 (eg. 0.02% Tween 20 ™)

4. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, eg. 0.02% Tween 20 ™, 15 mM NaN_3

5. Elution solution: 2 mM EDTA, pH 8.0 , 15 mM NaN_3, eg. 0.02% Tween 20 ™

6. Reconditioning solution: 0.1 M NaOH , eg. 0.02% Tween 20 ™

7. Storage buffer Oligo $(dT)_{25}$: 250 mM Tris-Hcl, pH 8, 20 mM EDTA, 0.1% Tween-20, 15 mM NaN_3

Example of the reagents used for a mRNA purification

1. Binding buffer: 20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA, 15 mM NaN_3 , eg. 0.02% Tween 20 ™

2. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 mM LiCl, 1 mM EDTA, 15 mM NaN_3, eg. 0.02% Tween 20 ™

5. Elution solution: 2 mM EDTA, pH 8.0 , 15 mM NaN_3, eg. 0.02% Tween 20 ™

Example of reagents used for RNA isolation

1. 4 M guanidium isothiocyanate, 25 mM sodium citrate pH 7.0, 0.5% N-lauryl sarcosine, 0.01 M β-mercaptoethanol

Example of reagents used for a DNA direct purification

1. Particle suspension in Lysis buffer (eg. 50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, 1% 2-mercaptoethanol; 50 mM KCl, 10 - 20 mM Tris-HCl, 2.5 mM $MgCl_2$, pH 8.3, 0.5 Tween 20 ™, 100 µg/ml Proteinase K; 100 mM Tris-HCl, pH 8.5, 5 mM EDTA, 1% SDS, 500 µg/ml Proteinase K) containing 15 mM NaN_3

2. Washing buffer containing 15 mM NaN_3 and eg. 0.02% Tween 20 ™

3. Resuspension buffer containing 15 mM NaN_3 and eg. 0.02% Tween 20 ™

Example of the purification process of PCR products by a magnetic particle processor at room temperature

The reagents are dispensed into a subsequent wells of a plate.

Example of a reagent configuration:

Well 1. Sample (biotinylated DNA, PCR amplicons)
Well 2. Streptavidin coated magnetic particles in washing buffer
Wells 3 - 5. Washing buffer
Well 6. NaOH
Well 7. TE buffer
Well 8. Distilled water

Example of processing steps:

Well 2. Mixing, washing and collecting of particles, moving of them into well 3
Well 3. Washing of particles, moving of them into well 4
Well 4. Washing of particles, moving of them into well 1
Well 1. Sample incubation 10', moving of particles into well 4
Well 4. Washing of particles, moving of them into well 5
Well 5. Washing of particles, moving of them into well 6
Well 6. Incubation 5' in melting solution, moving of particles into well 4
Well 4. Washing of particles, moving of them into well 5
Well 5. Washing of particles, moving of them into well 7
Well 7. Rinsing of particles, moving of them into well 8
Well 8. Releasing of particles

The effect of surface tension releasing agent (STRA) in collecting and releasing steps of magnetizable particles

Streptavidin coated magnetic particles (sizes: Scigen streptavidin 3 µm; Scigen; SPHERO™ streptavidin 4 - 4.5µm, Spherotec, Dynabeads M-280 streptavidin 2.8 µm, Dynal) were saturated with biotinylated alkaline phosphatase (Calbiochem, San Diego, CA) for 1 h at +37 °C. Saturated particles were first washed to remove the unbound alkaline phosphatase and were then used to examine the effect of STRA in collecting and releasing steps of a magnetic particle processor. The instrument settings of these examples were adjusted from 20 µl to 200 µl and the capacity range of the processor was 1 - 24 samples per run. The processor utilized a rod magnet (cylindrical NdFeB axially magnetized, length 2 mm, width 3 mm) in polypropene tube (outer width 4.5 mm).

Briefly, the particles were processed by releasing and collecting them from well to well so that the whole process comprised of 10 steps. The amount of particles, which remained into the wells after the collection, was estimated with alkaline phosphatase assay. Samples (10 µl) from each well were transferred to an empty microtitration plate (round-bottomed wells, width 6.5 mm).

In this assay alkaline phosphatase saturated particles (0.016 µg - 1 µg particles / 10 µl diluent) were used as standards. Into the wells containing 10 µl samples and standards were added 100 µl pNPP-substrate diluted in diethanolamine (DEA) buffer (Labsystems). The substrate was incubated for 15 minutes at +37 °C with continuous shaking (900 rpm) in Labsystems iEMS Incubator/Shaker. The reaction was stopped by adding 100 µl 1M NaOH into each well and the absorbances at 405 nm were measured by photometer (Labsystems Multiskan).

The amount of remaining particles was determined from a linear standard curve and finally results were expressed as percentage of initial amount of particles (0.2 mg/well).

In Fig 1. is shown the effect of detergent (Tween 20 ™) in different concentrations. The degree of remaining particles (Scigen streptavidin) were over 3% / well, when surface tension releasing agent was not added into the collecting and releasing buffer. When the detergent concentration was ≥ 0.00125%, the particles were collected efficiently.

In Fig 2. is shown the effect of salt and saccharose in collecting and releasing buffer. By adding these components into the buffer, the collection of particles (Scigen streptavidin) was more efficient.

In Fig 3. is shown the effect of a protein (casein) which was improving the collecting steps of particles (SPHERO™ streptavidin) in some degree.

In Fig. 4 is shown the effect of detergent (Tween 20 ™) when the magnetic particles of different suppliers were used.

Claims

1. A process for the purification of a substance, wherein
- material containing the substance, and magnetic particles coated or treated with a reagent which binds the particles to the substance are dispensed in a first medium,
- a binding reaction is let to take place, in which reaction the substance is bound to the particles, and
- a magnetic probe is pushed into the medium, whereby the particles adhere to the probe, and the probe together with the particles and the substance bound to them is transferred to a second medium, and if desired, separated from the second medium and transferred to a third medium,
characterized in that
- a surface tension releasing agent is dispensed at least on of the mediums, preferably at least to the first medium, and most preferably to all mediums, before the probe and the particles are transferred from it.

2. A method according to claim 1, wherein the surface releasing compound is a tenside, alcohol, protein, or a salt or carbohydrate.

3. A method according to claim 1 or 2, wherein the surface tension releasing compound is a tenside, such as a detergent.

4. A method according to claim 3, wherein the concentration of the tenside is 0.001 - 0.5% (w/v), preferably 0.005 - 0.1% (w/v), and most preferably 0.01 - 0.05% (w/v).

5. A method according to claim 1 or 2, wherein the surface tension releasing compound is a protein.

6. A method according to claim 5, wherein the concentration of the protein is 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v).

7. A method according to claim 1 or 2, wherein the surface tension releasing compound is a salt.

8. A method according to claim 7, wherein the concentration of the salt is 0.1 - 10 M, preferably 0.1 - 7 M.

9. A method according to any of claims 1-8 for the prurification of cells, viruses, subcellular organelles, proteins, or nucleic acid materials.

10. A method according to claim 9 for the prurification of nucleic acid materials.

11. A method according to any of claims 1-10, wherein the size of the magnetic particles is less than 50 μm, preferably 0.1 - 10 μm, and most preferably 1 - 5 μm.

12. A method according to any of claims 1-11, wherein the concentration of the magnetic particles is 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.

13. A method for separating magnetic particles by means of a magnetic probe from a medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are separated.

14. A method for improving the adherence of magnetic particles from a liquid medium to a magnetic probe to be pushed into the medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are adherted to the probe.

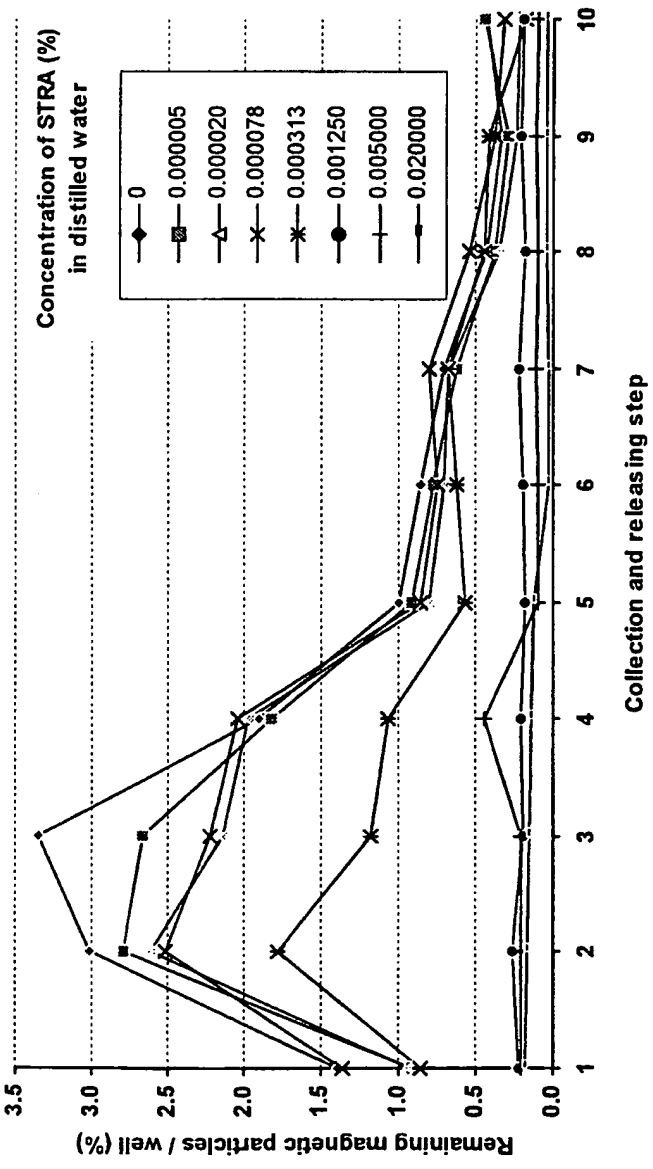
Concentration of STRA (%)
in distilled water
0
0.000005
0.000020
0.000078
0.000313
0.001250
0.005000
0.020000
Remaining magnetic particles / well (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step

Fig. 1

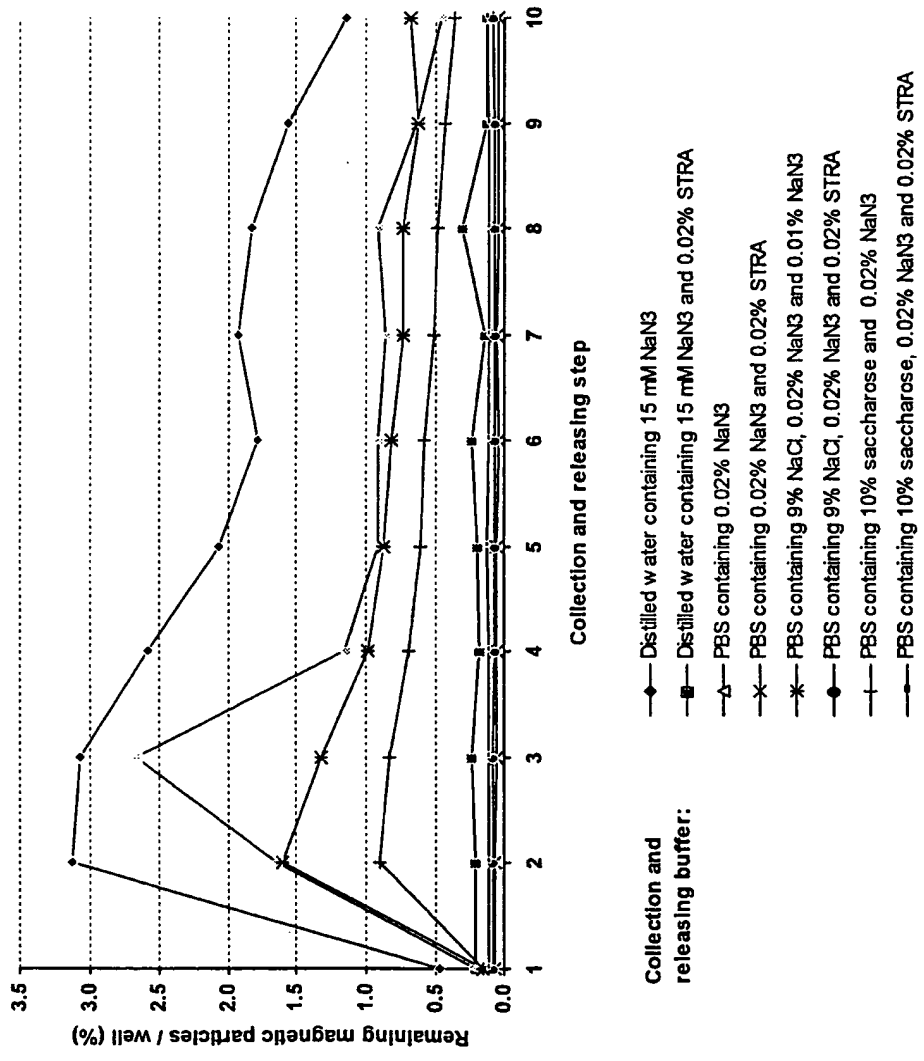
Remaining magnetic particles / well (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
Collection and releasing buffer:
Distilled water containing 15 mM NaN3
Distilled water containing 15 mM NaN3 and 0.02% STRA
PBS containing 0.02% NaN3
PBS containing 0.02% NaN3 and 0.02% STRA
PBS containing 9% NaCl, 0.02% NaN3 and 0.01% NaN3
PBS containing 9% NaCl, 0.02% NaN3 and 0.02% STRA
PBS containing 10% saccharose and 0.02% NaN3
PBS containing 10% saccharose, 0.02% NaN3 and 0.02% STRA

Fig. 2

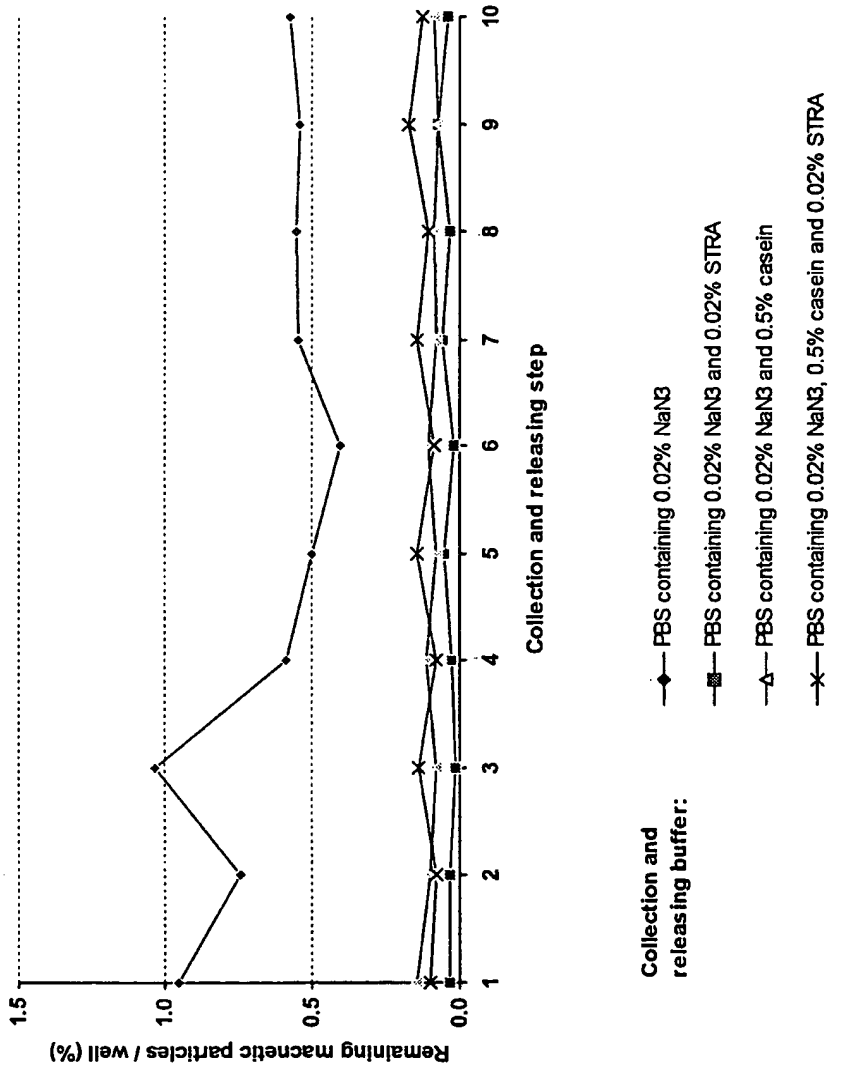
Remaining macnetic particles / well (%)
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
Collection and releasing buffer:
PBS containing 0.02% NaN3
PBS containing 0.02% NaN3 and 0.02% STRA
PBS containing 0.02% NaN3 and 0.5% casein
PBS containing 0.02% NaN3, 0.5% casein and 0.02% STRA

Fig. 3

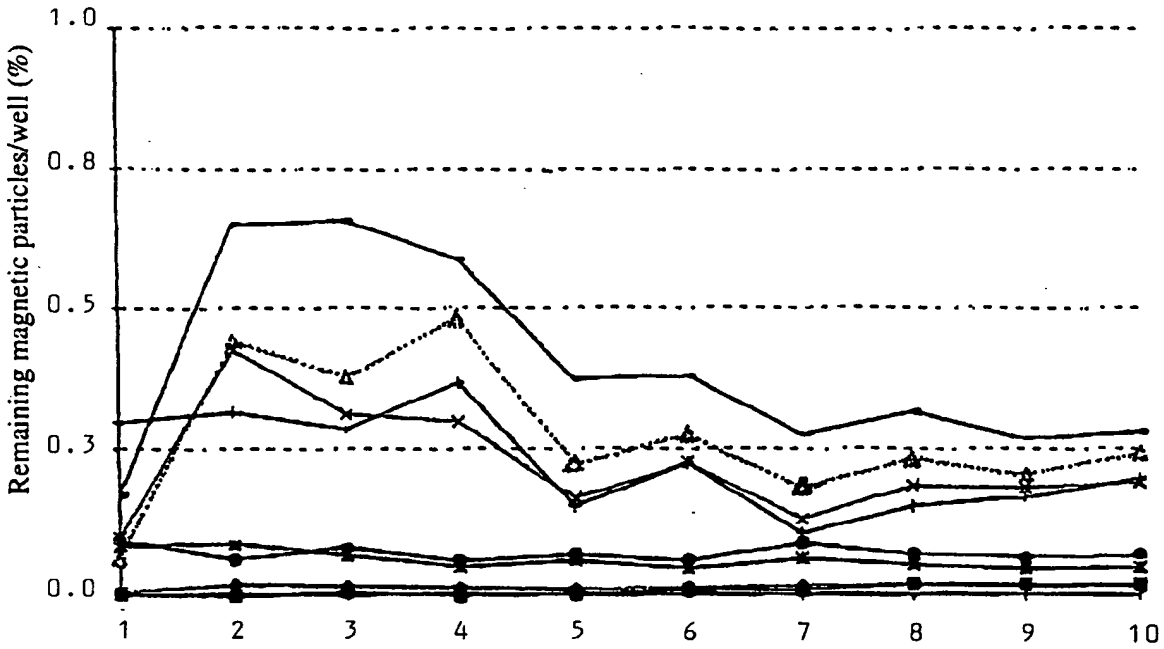
Remaining magnetic particles/well (%)
1.0
0.8
0.5
0.3
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
DYNAL (PBS. 0.01% NaN3, 0.5% Tween 20)
DYNAL (PBS. 0.01% NaN3, 0.02% Tween 20)
DYNAL (PBS. 0.01% NaN3, 0.0005% Tween 20)
DYNAL (PBS. 0.01% NaN3)
SPHEROTEC (PBS. 0.01% NaN3, 0.5% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3, 0.02% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3, 0.0005% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3)

Fig. 4

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Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9618731 A2 (DYNAL A/S), 20 June 1996 (20.06.96), see abstract; page 6, line 23 - page 12, line 9 --	1-14
X	US 5705628 A (TREVOR HAWKINS), 6 January 1998 (06.01.98), see abstract; column 1, line 24 - column 2, line 60; column 5, line 29 - column 6, line 27 --	1-14
A	WO 9831840 A1 (PROMEGA CORPORATION), 23 July 1998 (23.07.98), see abstract; page 7, lines 9-21; page 8, lines 2-7; page 8, line 30 - page 9, line 12; page 12, line 1 - page 13, line 8; page 17, line 20 - page 21, line 12 --	1-14

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 May 2000	17 -05- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00031

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Microbiology, Volume 140, 1994, Anil Wipat et al, "Monoclonal antibodies for Streptomyces lividans and their use for immunomagnetic capture of spores from soil", page 2067 - page 2076, see abstract --	1-14
A	WO 9612959 A1 (LABSYSTEMS OY), 2 May 1996 (02.05.96), see abstract -- --------	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00031

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00031

According to PCT Rule 13.1 and 13.2, the international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). In order to achieve unity of invention the single general inventive concept must involve a "special technical feature" i.e. a technical feature that is common to the inventions and defines a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

With regard to the documents cited in the International Search Report, the present application is considered to consist, à posteriori, of the following inventions:

1. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a tenside. This invention is disclosed in claims 1-4 and 9-14 (partially).

2. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a protein. This invention is disclosed in claims 1-2, 5-6 and 9-14 (partially).

3. A process for the purification of a substance characterised in that magnetic particles coated with a reagent are used; a binding reaction takes place; a magnetic probe is pushed into the medium in order to transfer the particles from one solution to another; a surface tension releasing agent is dispersed in the medium before the probe and the particles is transferred from it. In the first invention the surface releasing compound is a salt. This invention is disclosed in claims 1-2, 7-8 and 9-14 (partially).

It is considered that all the inventions could be searched within one fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/FI 00/00031

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9618731 A2	20/06/96	AU 706211 B	10/06/99
		AU 4182996 A	03/07/96
		CA 2207608 A	20/06/96
		EP 0796327 A	24/09/97
		GB 9425138 D	00/00/00
		JP 11501504 T	09/02/99
US 5705628 A	06/01/98	IL 115352 D	00/00/00
		US 5898071 A	27/04/99
		WO 9609379 A	28/03/96
WO 9831840 A1	23/07/98	AU 6647598 A	07/08/98
		CA 2249393 A	23/07/98
		EP 0895546 A	10/02/99
		JP 11509742 T	31/08/99
WO 9612959 A1	02/05/96	EP 0788602 A	13/08/97
		FI 944938 D	00/00/00
		FI 971666 A	18/04/97
		JP 10508099 T	04/08/98
		NO 971805 A	18/04/97
		US 5942124 A	24/08/99